

Exhibit 3

1/2 Digest FT cDNA 3, 10, 14 w/ KpnI/HindIII
use previous prep as control (-)

Expected Size

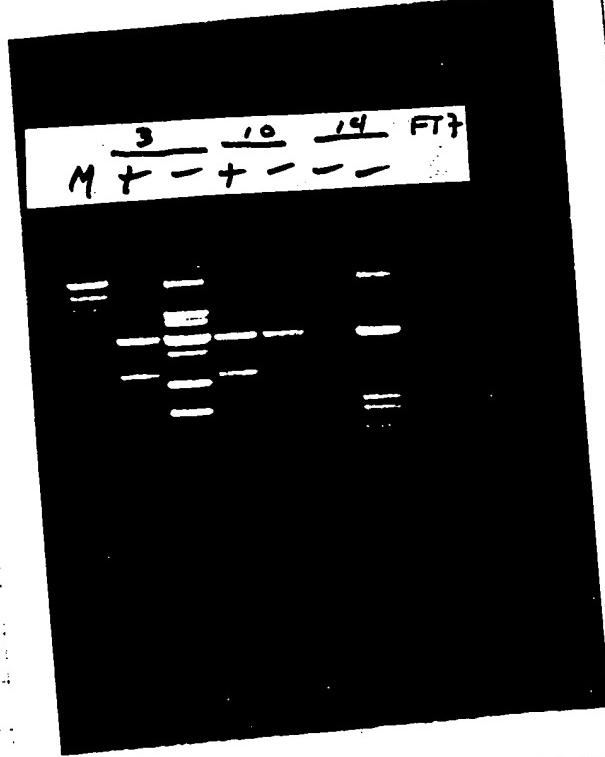
(3) Since 4 units S. (-)

3159	3159
1573	1366
625	782

10	3159	3159
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1573	1121
650	832
380	650

14	3159	3159
1573	945	
650	832	
204	650	



Major problem w/ #14

Some how samples got mixed up

6.0 Bend & elutant - streak for single colonies

A B : E

Also mini prep from original culture

Digest w/ KpnI/HindIII 0/14

1/23 Run gel of Digests

Clearly Sample A which was grown as mate prep is
in the wrong orientation

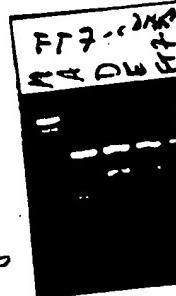
Start 0/14 of D : E to mini prep before

start of 500ml culture

For mini prep - FT cDNA 14-D : E

Digest w/ KpnI/HindIII

run on gel - Both are fine
use D for mate



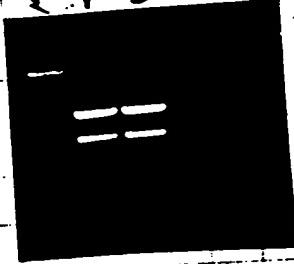
MDE

7/25 Plasmid isolation of FT7, cDNA 14 - D up to Banding
 - do 500mls in 2 250 sets, end w/ 2 tubes to band
 7/26 Pull Bands - double band 1 prep - 6 hrs during the day

7/27 Digest FT7.cDNA14 w/ Kpn I (H and I)

- (1) - Single band
- (2) Double Banding
- (3) FT7.cDNA14 antisense

FT7.14 SENSE FT7
1 2 M13 ANTI



Check Absorbance / concentration of

Samples	$\frac{260}{280}$	$\frac{260}{280}$	260/280
(1) .127	.062	.63 ng/ μ l	2.0
(2) .143	.065	.71 ng/ μ l	2.3

Sequence
 FT7, cDNA 3

T7

cDNA-14

T7

885D

9007

8874

Digoxin Mouse FT4 260 280 260/280

PSC7 genomic .085 .045

WT Coding .106 .060

Digoxin cDNA 3

Kpn I cDNA 10

Hind III cDNA 11

cDNA 11

M FT7.14 SENSE
FT4 cDNA 6
FT9 cDNA 3
FT11 cDNA 10
FT7 cDNA 11



8/4 FACS Analyses of Transfected cells w/ following vectors

pCDNA I

FT7, 12a 2b, 3
1, 2b, 3
1, 3

cDNA 3

cDNA 10

cDNA 14

2 plates/Vector - D. side

FACS Fms

yubo 5mls

CAT 3mls

FTaasy 5mls (7.5ml)

(100µl)

2nd antibody

IgM 2.5mls

12.5 / 2.5mls

IgG 1.5
6.0 / 1.5mls

Antibodies

IgM H - black 1:100 /

IgM LF green 1:1000

IgM SLX 1:200

IgG LF red 1:500

IgG SLA blue 1:500

Results are

H. all neg

LF - all neg

SLX, pD (-), 1, 2a 2b, 3 (+), 1, 2b, 3 (-), 1, 3 (-), cDNA 3 (-), 10 (+), 14 (+)

LA all neg

, LA all neg

9/3 Sperm 12 pmml KG FT4

6451 6080

2470 6199

6374 6087

6306 6086

6203 6085

5721 5671

8/9 - Running gel of above samples
Also sperm

5728	6084	7213
5731	5732	6082
5662	5722	6201
		6200

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8/10 Screening of 8/9 samples
Sequence

FT4 6079

6202

6307

6373

cDNA 10 715

946

CDNA 14

T7

8850

8807

8874

Protein assay of FACS Samples, Also CAT assay

pcDNA

FT7, 1,2a2b,3

FT7, 1,2b,3

FT7, 1,3

cDNA 3

cDNA 10

cDNA 14

BSA Biot Protein

0 1.00 116 108

1 .208 .225 .214

2 .369 .383 .376

4 .691 .673 .682

8 1.215 1.230 1.222

16

Sue's Sample

pcDNA .292 .294 .293

1,2a2b,3 -332 .330 .33

1,2b,3 .298 .343 .32

1,3 .369 .372 .35

cDNA 3 .363 .379 .3

cDNA 10 .306 .298 .3

cDNA 14 .225 .253 .3

FACS Results

Only stain w/ SHx
1,2a2b,3 23.6%

1,2b,3 24.6%

cDNA 10 14.9%

cDNA 14 8.0%

Micro BCA Protein Assay

Reagent mix	MC	MB	MA
Per assay tube (ml)	0.01	0.24	0.25
Cocktail for Tubes			

Incubate 1 h at 60°C and cool to room temp.

Since the color development has no end point, all tubes must be heated and cooled at the same time

1 mg/ml BSA (l)	Water (l)	Reagent (l)	Abs. 562	
0.0	500.0	500.0	Blank	Slope = 0.0734
1.0	499.0	500.0	0.108	Y intercept = 0.0656
2.0	498.0	500.0	0.214	X intercept = -0.8940
4.0	496.0	500.0	0.376	R = 0.9985
8.0	492.0	500.0	0.682	
16.0	484.0	500.0	1.222	

8/10 Screening of FT4 9/10 Samples

Sample	l in assay	Water (l)	Reagent (l)	Abs. 562	mg protein/ml
pcDNAI	5.00	495.00	500	0.293	0.62
FT7 1,2a2b,3	5.00	495.00	500	0.333	0.73
FT7 1,2b,3	5.00	495.00	500	0.320	0.69
FT7 1,3	5.00	495.00	500	0.370	0.83
cDNA 3	5.00	495.00	500	0.371	0.83
cDNA 10	5.00	495.00	500	0.302	0.64
cDNA 14	5.00	495.00	500	0.239	0.47

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8/11 CAT Assay (FT7) samples
2.5 μl of cell extract

Control

pcDNA

1,2a,2b,3

1,2b,3

1,3

CDNA 3

CDNA 10

CDNA 14

Cocktail	15
3/4 Chlce	300
Tris, 2M pH 10	25
But Co A	75
H ₂ O	300
	<u>50</u> /tube

CAT

Vector	Counts/5μl 8/12/94	Incorporated Counts (.95)	Total Counts	Total Counts Incorporated
pcDNA1	11,349	9,169	8,063	238,189
FT7 1,2a2b,3	11,181	11,441	27,211	250,431
FT7 1,2b,3	11,772	11,826	37,541	272,981
FT7 1,3	11,215	11,690	23,076	28,706
CDNA 3	11,834	11,206	33,885	39,096
CDNA 10	12,017	11,312	30,066	33,165
CDNA14	11,079	11,570	44,133	40,529
Control			10,354	424
Protein Conc. (ug/2.5μl)	Total Counts Inc. Blkg	% INC/hr	% INC/hr/ug	Mean CAT Activity
pcDNA1	1.55	9,249	8,063	3.92
FT7 1,2a2b,3	1.83	28,219	22,649	11.27
FT7 1,2b,3	1.72	39,093	42,401	14.32
FT7 1,3	2.07	23,867	29,793	9.65
CDNA 3	2.07	35,244	40,730	13.03
CDNA 10	1.60	31,224	34,487	11.55
CDNA14	1.17	46,032	42,238	17.32

8/11 Assemble Data of FT7 FACS, CAT assay / give to Julie
Work on FT4 samples

9/5 7 days sequencing on Troubleshooting FT4 samples

6451	6200
6378	6079
6306	6307
6203	1897
5721	1898
	1899

9/6 Sequencing gel of 8/15 samples (FT4) Tammie's gel
Probe Deoxyribonucleic acid w/ GAP probe
To check condition of RFLP

9/6 The 7 days technique didn't resolve all of the capsidions
Try a Terminal transferase technique
Run standard sequencing 14n, agar extension made
Heat tubes (A, G, C, T) for 1.5 mins 100°C
Hold on ice 10 mins, Prepare TdT/dNTP cocktail
Add to tubes, 37°C 30 min
Add Stop